Complete Summary

GUIDELINE TITLE

Laboratory support for the diagnosis and monitoring of thyroid disease.

BIBLIOGRAPHIC SOURCE(S)

National Academy of Clinical Biochemistry (NACB). NACB: laboratory support for the diagnosis and monitoring of thyroid disease. Washington (DC): National Academy of Clinical Biochemistry (NACB); 2002. 125 p. [495 references]

COMPLETE SUMMARY CONTENT

SCOPE

DISEASE/CONDITION(S)

Thyroid disease

GUIDELINE CATEGORY

Diagnosis
Evaluation

CLINICAL SPECIALTY

Endocrinology
Family Practice
Internal Medicine
Pathology
Pediatrics

INTENDED USERS

Clinical Laboratory Personnel
Physicians

GUIDELINE OBJECTIVE(S)

- To provide recommendations on the technical aspects of thyroid testing and the performance criteria needed for optimal clinical utility of thyroid tests
- To give both clinical laboratory scientists and practicing physicians an overview regarding the current strengths and limitations of those thyroid tests most commonly used in clinical practice

TARGET POPULATION

Patients with thyroid disease

INTERVENTIONS AND PRACTICES CONSIDERED

Diagnosis/Monitoring

Laboratory tests performed on serum by either manual or automated methods that employ specific antibodies.

1. Total thyroxine (TT4) and total triiodothyronine (TT3) measurement
2. Free thyroxine (FT4) and free triiodothyronine (FT3) estimate tests
3. Thyrotropin/thyroid stimulating hormone (TSH) measurement

Selective Testing

1. Thyroid autoantibody tests (autoimmune thyroid disease [AITD])
    - Thyroid peroxidase antibodies (TPOAb)
    - Thyroglobulin antibodies (TgAb)
    - Thyrotropin receptor antibodies (TRAb)
2. Thyroglobulin (Tg)
3. Calcitonin (CT) and ret Proto-oncogene
4. Urinary iodide measurement using dry or wet-ash techniques
5. Thyroid fine needle aspiration (FNA) and cytology

Screening

Congenital hypothyroidism (CH) high volume screening conducted by laboratories with experience in automated immunoassay procedures, information technology with computer back-up, and appropriately trained staff.

MAJOR OUTCOMES CONSIDERED

Sensitivity, specificity, and precision of laboratory tests used in the diagnosis and monitoring of thyroid disease

METHODOLOGY

METHODS USED TO COLLECT/SELECT EVIDENCE

Searches of Electronic Databases

DESCRIPTION OF METHODS USED TO COLLECT/SELECT THE EVIDENCE

Not stated

NUMBER OF SOURCE DOCUMENTS

Not stated

METHODS USED TO ASSESS THE QUALITY AND STRENGTH OF THE EVIDENCE

Expert Consensus

RATING SCHEME FOR THE STRENGTH OF THE EVIDENCE

Not applicable

METHODS USED TO ANALYZE THE EVIDENCE

Review of Published Meta-Analyses
Systematic Review

DESCRIPTION OF THE METHODS USED TO ANALYZE THE EVIDENCE

Not stated

METHODS USED TO FORMULATE THE RECOMMENDATIONS

Expert Consensus

DESCRIPTION OF METHODS USED TO FORMULATE THE RECOMMENDATIONS

Consensus recommendations were made throughout the guideline monograph. The consensus level was > 95%, unless otherwise indicated.

RATING SCHEME FOR THE STRENGTH OF THE RECOMMENDATIONS

Not applicable

COST ANALYSIS

A formal cost analysis was not performed and published cost analyses were not reviewed.

METHOD OF GUIDELINE VALIDATION

Peer Review

DESCRIPTION OF METHOD OF GUIDELINE VALIDATION

Expert reviewers are acknowledged by name in Appendix A of the original guideline document.

RECOMMENDATIONS

MAJOR RECOMMENDATIONS

Please Note: A list of abbreviations is provided at the end of the "Major Recommendations" field.

Pre-analytic Factors

Guideline 1. General Guidelines for Laboratories and Physicians

- Laboratories should store (at 4-8Â°C) all serum specimens used for thyroid testing for at least one week after the results have been reported to allow physicians time to order additional tests when necessary.
- Specimens from differentiated thyroid cancer (DTC) patients sent for serum thyroglobulin (Tg) measurement should be archived (at -20Â°C) for a minimum of six months.

Guideline 2. Thyroid Testing for Ambulatory Patients

- Patients with stable thyroid status: When thyroid status is stable and hypothalamic-pituitary function is intact, serum thyroid stimulating hormone (TSH) measurement is more sensitive than free thyroxine (FT4) for detecting mild (subclinical) thyroid hormone excess or deficiency. The superior diagnostic sensitivity of serum TSH reflects the log/linear relationship between TSH and FT4 and the exquisite sensitivity of the pituitary to sense free T4 abnormalities relative to the individual's genetic free T4 set-point.
- Patients with unstable thyroid status: Serum FT4 measurement is a more reliable indicator of thyroid status than TSH when thyroid status is unstable, such as during the first 2-3 months of treatment for hypo- or hyperthyroidism. Patients with chronic, severe hypothyroidism may develop pituitary thyrotroph hyperplasia that can mimic a pituitary adenoma, but resolves after several months of levothyroxine (L-T4) replacement therapy. In hypothyroid patients suspected of intermittent or non-compliance with L-T4 replacement therapy, both TSH and FT4 should be used for monitoring. Non-compliant patients may exhibit discordant serum TSH and FT4 values (high TSH/high FT4) because of persistent disequilibrium between FT4 and TSH.

Guideline 3. Thyroid Testing of Infants and Children

The hypothalamic-pituitary-thyroid axis matures throughout infancy until the end of puberty.

- Both TSH and FT4 concentrations are higher in children, especially in the first week of life and throughout the first year. Failure to recognize this could lead to missing and/or under-treating cases of congenital hypothyroidism.
- Age-related normal reference limits should be used for all tests (see Table 3 in the original guideline document).

Guideline 4. Thyroid Testing of Pregnant Patients

Mounting evidence suggests that hypothyroidism during early pregnancy has a detrimental effect on fetal outcome (fetal wastage and lower infant IQ).

- Pre-pregnancy or first trimester screening for thyroid dysfunction using serum TSH and thyroperoxidase autoantibodies (TPOAb) measurements is important both for detecting mild thyroid insufficiency (TSH >4.0 mIU/L) and for assessing risk for post-partum thyroiditis (elevated TPOAb).
- Initiation of levothyroxine (L-T4) therapy should be considered if the serum TSH level is >4.0 mIU/L in the first trimester of pregnancy.
- A high serum TPOAb concentration during the first trimester is a risk factor for post-partum thyroiditis.
- Serum TSH should be used to assess thyroid status during each trimester when pregnant patients are taking L-T4 therapy, with more frequent measurement if L-T4 dosage is changed.
- Trimester-specific reference intervals should be used when reporting thyroid test values for pregnant patients.
- Total thyroxine (TT4) and triiodothyronine (TT3) measurements may be useful during pregnancy if reliable FT4 measurements are not available, as long as the reference ranges are increased by 1.5-fold relative to non-pregnant ranges.
- Free triiodothyronine (FT3) and free thyroxine (FT4) reference ranges in pregnancy are method-dependent and should be established independently for each method.
- Measurement of serum thyroglobulin (Tg) in differentiated thyroid cancer (DTC) patients during pregnancy should be avoided. Serum Tg rises during normal pregnancy and returns to baseline levels post-partum. This rise is also seen in pregnant DTC patients with remnant normal thyroid or tumor tissue present and is not necessarily a cause for alarm.

Guideline 5. Patients taking Amiodarone Medication

Amiodarone therapy can induce the development of hypo- or hyperthyroidism in 14-18% of patients with apparently normal thyroid glands or with preexisting abnormalities.

- Pretreatment. Thorough physical thyroid examination together with baseline TSH and TPOAb. FT4 and free triiodothyronine (FT3) tests are only necessary if TSH is abnormal. Positive TPOAb is a risk factor for the development of thyroid dysfunction during treatment.

- First 6 months. Abnormal tests may occur in the first six months after initiating therapy. TSH may be discordant with thyroid hormone levels (high TSH/highT4/low T3). TSH usually normalizes with long-term therapy if patients remain euthyroid.
- Long-term follow-up. Monitor thyroid status every 6 months with TSH. Serum TSH is the most reliable indicator of thyroid status during therapy.
- Hypothyroidism. Preexisting Hashimoto's thyroiditis and/or TPOAb-positivity is a risk factor for developing hypothyroidism at any time during therapy.
- Hyperthyroidism. Low serum TSH suggests hyperthyroidism. T3 (total and free) usually remains low during therapy but may be normal. A high T3 is suspicious for hyperthyroidism.

Two types of amiodarone-induced hyperthyroidism may develop during therapy, although mixed forms are frequently seen (20%). Distinction between two types is often difficult. Decreased flow on color flow doppler and elevated interleukin-6 suggests Type II. Direct therapy at both Type I and II if etiology is uncertain.

Type I = Iodine-induced. Recommended treatment = simultaneous administration of thionamides and potassium perchlorate (if available). Some recommend iopanoic acid before thyroidectomy. Most groups recommend that amiodarone be stopped. Seen more often in areas of low iodine intake. However, in iodine-sufficient areas, radioiodine uptakes may be low precluding radioiodine as a therapeutic option. In iodide-deficient regions, uptakes may be normal or elevated.

> Type a: Nodular goiter. More common in iodine-deficient areas, i.e. Europe.
>
> Type Ib: Graves' disease. More common in iodine-sufficient areas, i.e. United States.

Type II = amiodarone-induced destructive thyroiditis--a self-limiting condition. Recommended treatment = glucocorticoids and/or beta-blockers if cardiac status allows. When hyperthyroidism is severe, surgery with pre-treatment with iopanoic may be considered. Radioiodine uptake is typically low or suppressed. Type II is more commonly seen in iodine-sufficient areas.

Guideline 6. For Testing of Hospitalized Patients with Non Thyroidal Illness

- Acute or chronic nonthyroidal illness (NTI) has complex effects on thyroid function tests. Whenever possible, diagnostic testing should be deferred until the illness has resolved, except when the patient's history or clinical features suggest the presence of thyroid dysfunction.
- Physicians should recognize that some thyroid tests are inherently non-interpretable in severely sick patients, or patients receiving multiple medications.
- TSH in the absence of dopamine or glucocorticoid administration, is the more reliable test for NTI patients.
- Estimates of free or total T4 in NTI should be interpreted with caution, in conjunction with a serum TSH measurement. Both T4 + TSH measurements are the most reliable way for distinguishing true primary thyroid dysfunction

(concordant T4/TSH abnormalities) from transient abnormalities resulting from NTI per se (discordant T4/TSH abnormalities).

- An abnormal FT4 test in the setting of serious somatic disease is unreliable, since the FT4 methods used by clinical laboratories lack diagnostic specificity for evaluating sick patients.
- An abnormal FT4 result in a hospitalized patient should be confirmed by a reflex TT4 measurement. If both TT4 and FT4 are abnormal (in the same direction) a thyroid condition may be present. When TT4 and FT4 are discordant, the FT4 abnormality is unlikely due to thyroid dysfunction and more likely a result of the illness, medications or an artifact of the test.
- TT4 abnormalities should be assessed relative to the severity of illness, since the low TT4 state of NTI is typically only seen in severely sick patients with a high mortality rate. A low TT4 concentration in a patient not in intensive care is suspicious for hypothyroidism.
- A raised total or free T3 is a useful indicator of hyperthyroidism in a hospitalized patient, but a normal or low T3 does not rule it out.
- Reverse T3 testing is rarely helpful in the hospital setting, because paradoxically normal or low values can result from impaired renal function and low binding protein concentrations. Furthermore, the test is not readily available in most laboratories.

Guideline 7. Investigation of Discordant Thyroid Test Results

Discordant thyroid test results can result from technical interference or rare clinical conditions:

- Technical Interference: Technical interference can sometimes be detected by measuring the specimen with a different manufacturer's method, since the magnitude of most interferences is method-dependent. Alternatively, non-linearity in dilutions of the specimen may indicate a technical interference with TT4, TT3 or TSH measurements. Note: a 100-fold dilution of a "normal" serum theoretically causes insignificant reduction (<2%) in the FT4 concentration. It is not recommended to dilute the FT4 and FT3 tests used by clinical laboratories because such tests are binding protein dependent and do not give linear dilution responses.
- Rare Clinical Conditions: Unexpectedly abnormal or discordant thyroid test values may be seen with some rare, but clinically significant conditions such as central hypothyroidism, TSH-secreting pituitary tumors, thyroid hormone resistance, or the presence of heterophilic antibodies (HAMA) or thyroid hormone (T4 and/or T3) autoantibodies.

Guideline 8. Guidelines for Interpreting Thyroid Test Results

- For diagnostic testing (case-finding) thyroid test results are typically reported together with a "normal" reference interval that reflects between-person variability.
- The "normal" reference interval does not indicate the magnitude of difference between test results that constitutes a clinically significant change.

  Analytical variability together with between-person and within-person estimates of biological variability suggests that the magnitude of difference in

thyroid test values that would be clinically significant when monitoring a patient's response to therapy are:

TT4 = 28 (2.2) nmol/L (micrograms/dL)

FT4 = 6 (0.5) pmol/L (ng/dL)

TT3 = 0.55 (35) nmol/L (ng/dL)

FT3 = 1.5 (0.1) pmol/L (ng/dL)

TSH = 0.75 mIU/L

Tg = 1.5 micrograms/L (ng/mL)

Thyroid Tests for the Clinical Biochemist and Physician

Total Thyroxine (TT4) and Total Triiodothyronine (TT3) Methods

Guideline 9. For Manufacturers Developing Total Thyroxine (TT4) and Total Triiodothyronine (TT3) Methods

Method biases should be reduced by:

- The development of L-T4 and L-T3 reference preparations and establishing international reference methods.
- Ensuring that instruments are not sensitive to differences between human serum and the calibrator matrix.
- Ensuring that during the test process, the amount of thyroid hormone released from serum binding proteins is the same as that released in the presence of the calibrator diluent.

Guideline 10. Serum Total T4 (TT4) and Total T3 (TT3) Measurements

Abnormal serum TT4 and TT3 concentrations are more commonly encountered as a result of binding protein abnormalities and not thyroid dysfunction.

- Free T4 estimate tests (FT4) are preferred over TT4 measurement when thyroxine binding globulin (TBG) concentration is abnormal. However, FT4 tests may be diagnostically inaccurate when the affinity of TBG is altered or abnormal T4-binding proteins are present.
- Total hormone assays (TT4 and TT3) should remain readily available to evaluate discordant free hormone tests.

Free Thyroxine (FT4) and Free Triiodothyronine (FT3) Estimate Tests

Guideline 11. Free Hormone Test Nomenclature

- The free hormone methods used by most clinical laboratories (indexes and immunoassays) do not employ physical separation of bound from free

hormone and do not measure free hormone concentrations directly! These tests are typically binding protein dependent to some extent and should more appropriately be called "Free Hormone Estimate" tests, abbreviated FT4E and FT3E.
- In general, Free Hormone Estimate tests overestimate the FT4 level at high protein concentrations and underestimate FT4 at low protein concentrations.

Index Methods: FT4I and FT3I

Guideline 12. Thyroid Hormone Binding Ratio (THBR) or "Uptake" Tests

- "Uptake" tests should be called "Thyroid Hormone Binding Ratio" tests, abbreviated THBR and include an indication of which hormone is used, i.e. THBR (T4) or THBR (T3).
- A T4 signal is preferred over T3 for THBR measurements, to better reflect T4 binding protein abnormalities.
- THBR values should be reported as a ratio with normal serum, the latter having an assigned value of 1.00.
- THBR calculations should be based on the ratio between absorbent counts divided by the total minus absorbent counts, rather than the ratio between absorbent counts and total counts.
- The THBR result should be reported in addition to the total hormone and free hormone index value.
- THBR tests should not be used as an independent measurement of thyroid status, but should be interpreted in association with a TT4 and/or TT3 measurement and used to produce free hormone estimates (FT4 or FT3 indexes).

Ligand Assays for FT4 and FT3 Estimation

Guideline 13. For Manufacturers Developing Free Hormone Estimate Tests

- Methods that do not physically separate bound from free hormone should extract no more than 1-2% of the total hormone concentration hormone off the binding proteins, so that the thermodynamic equilibrium is maintained as much as possible.
- Minimize dilution effects that weaken the influence of any endogenous inhibitors present in the specimen.
- Use serum calibrators containing known free hormone concentrations that behave in the assay in an identical manner to the patient specimens.
- Perform the test procedure at 37Â°C.

Guideline 14. Clinical Utility of Serum Free T3 Estimate Tests

Serum T3 measurement has little specificity or sensitivity for diagnosing hypothyroidism, since enhanced T4 to T3 conversion maintains normal T3 concentrations until hypothyroidism becomes severe. Patients with NTI or caloric deprivation typically have low total and free T3 values. Serum T3 measurements, interpreted together with FT4, and are useful to diagnose complex or unusual presentations of hyperthyroidism and certain rare conditions:

- A high serum T3 is often an early sign of recurrence of Graves' hyperthyroidism.
- The TT3/TT4 ratio can be used to investigate Graves' versus non-Graves' hyperthyroidism. Specifically, a high TT3/TT4 ratio (>20 ng/micrograms metric or >0.024 molar) suggests thyroidal stimulation characteristic of Graves' disease.
- Serum T3 measurement can be used to monitor the acute response to treatment for Graves' thyrotoxicosis.
- A high or paradoxically normal serum T3 may indicate hyperthyroidism in an NTI patient with suppressed TSH (< 0.01 mU/L).
- A high or paradoxically normal serum T3 may indicate amiodarone-induced hyperthyroidism.
- Patients with goiter living in areas of iodide deficiency should have FT3 measured in addition to TSH to detect T3 thyrotoxicosis caused by focal or multifocal autonomy.
- A high serum T3 is frequently found with congenital goiter, due to defective organification of iodide (thyroid peroxidase [TPO] defect) or defective synthesis of thyroglobulin.
- A high serum T3 usually precedes the iodide-induced thyrotoxicosis when patients have multinodular long-standing goiter.
- A high serum T3 is often seen with TSH-secreting pituitary tumors.
- A high serum T3 is often seen in thyroid hormone resistance syndromes that usually present without clinical hyperthyroidism.
- Serum T3 measurement is useful for monitoring compliance with L-T3 suppression therapy prior to ^{131}I scan for differentiated thyroid cancer (DTC).
- Serum T3 measurement is useful for distinguishing mild (subclinical) hyperthyroidism (low TSH/ normal FT4) from T3-toxicosis, sometimes caused by T3-containing health-foods.
- Serum T3 measurement is useful for investigating iodide deficiency (characterized by low T4/high T3).
- Serum T3 measurement can be useful during antithyroid drug therapy to identify persistent T3 excess, despite normal or low serum T4.
- Serum T3 measurement can be used to detect early recurrence of thyrotoxicosis after cessation of antithyroid drug therapy.
- Serum T3 measurement can be used to establish the extent of T3 excess during suppressive L-T4 therapy or after an intentional T4 overdose.

Guideline 15. Abnormal Thyroid Hormone Binding Proteins Effects on FT4 Tests

Binding protein abnormalities cause pre-analytical or analytical FT4 assay artifacts. Thyroid function should be assessed from the TSH-TT4 relationship when:

- The binding of assay tracer to albumin is abnormal (i.e. familial dysalbuminemic hyperthyroxinemia [FDH]).
- The patient is taking medications that displace T4 from TBG, i.e. phenytoin, carbamazepine or furosemide (frusemide).
- The patient has a critical or severe non-thyroidal illness.

Guideline 16. For Manufacturers: Assessment of FT4 Estimate Test Diagnostic Accuracy

- The diagnostic accuracy of the method should be tested using pedigreed specimens from ambulatory patients with the following binding protein disturbances:
    - TBG abnormalities (high estrogen & congenital TBG excess and deficiency)
    - Familial Dysalbuminemic Hyperthyroxinemia (FDH)
    - Increased Transthyretin (TTR) affinity
    - T4 and T3 Autoantibodies
    - Rheumatoid Factor
- Test the method for interference with normal serum specimens spiked with relevant concentrations of common inhibitors at concentrations that cause displacement of hormone from binding proteins in undiluted serum, effects which are lost after dilution i.e.:

  Furosemide (Frusemide) 30 micromoles

  Disalicylic acid 300 micromoles

  Phenytoin 75 micromoles

  Carbamazepine 8 micromoles

- List all known interferences with the magnitude and direction of resulting errors
- Document in-vitro heparin effects on non-esterified fatty acid (NEFA) generation during the assay incubation

Guideline 17. For Laboratories Performing FT4 and FT3 testing

- Physicians should have ready access to information on the effects of drugs and the diagnostic accuracy of the test used for assessing the thyroid status of patients with various binding protein abnormalities and severe illnesses.
- When requested by the physician, the laboratory should be prepared to confirm a questionable result by performing a total hormone measurement or by re-measuring FT4 by a reference method that physically separates free from bound hormone, such as direct equilibrium dialysis or ultrafiltration.
- Questionable results on specimens should be checked for interference by re-measurement made with a different manufacturer's method. (Send out to a different laboratory if necessary.)

Thyrotropin/Thyroid Stimulating Hormone (TSH)

Guideline 18. Investigation of Discordant Serum TSH Values in Ambulatory Patients

A discordant TSH result in an ambulatory patient with stable thyroid status may be a technical error. Specificity loss can result from laboratory error, interfering substances (i.e. heterophilic antibodies), or the presence of an unusual TSH isoform (see Guideline 7 above and Table 1 in the original guideline document). Physicians can request that their laboratory perform the following checks:

- Confirm specimen identity (i.e. have laboratory check for a switched specimen in the run).
- When TSH is unexpectedly high, ask the laboratory to re-measure the specimen diluted, preferably in thyrotoxic serum, to check for parallelism.
- Request that the laboratory analyze the specimen by a different manufacturer's method (send to a different laboratory if necessary). If the between-method variability for a sample is >50%, an interfering substance may be present.
- Once a technical problem has been excluded, biologic checks may be useful:
    - Use a thyrotropin-releasing hormone (TRH) stimulation test for investigating a discordant low TSH result, expect a 2-fold (≥4.0 mIU/L increment) response in TSH in normal individuals.
    - Use a thyroid hormone suppression test to verify a discordant high TSH level. Normal response to 1mg of L-T4 or 200 micrograms L-T3 administered p.o. is a suppressed serum TSH of more than 90% by 48 hours.

Guideline 19. Definition of Functional Sensitivity

Functional Sensitivity should be used to determine the Lowest Detection Limit of the assay.

TSH assay functional sensitivity is defined as a 20% between-run coefficient of variation (CV) determined by the recommended protocol (see Guideline 20).

Guideline 20. Protocol for TSH Functional Sensitivity & Between-Run Precision

Measure human serum pools covering the assay range in at least 10 different runs. The lowest pool value should be 10% above the detection limit and the highest pool value should be 90% of the upper assay limit.

- Carry-over should be assessed by analyzing the highest pool followed by the lowest pool.
- Use the same test mode as for patient specimens (i.e. singlicate or duplicate).
- The instrument operator should be blinded to the presence of test pools in the run.
- Runs should be spaced over a clinically representative interval (i.e. 6 to 8 weeks for TSH in an outpatient setting).
- Use at least two different lots of reagents and two different instrument calibrations during the testing period.
- When running the same assay on two similar instruments, blind duplicates should be run on each instrument periodically to verify correlation.

Guideline 21. For Laboratories Performing TSH Testing

Functional sensitivity is the most important performance criterion that should influence the selection of a TSH method. Practical factors such as instrumentation, incubation time, cost, and technical support though important, should be secondary considerations. Laboratories should use calibration intervals that optimize functional sensitivity, even if re-calibration needs to be more frequent than recommended by the manufacturer:

- Select a TSH method that has a functional sensitivity $\leq$0.02 mIU/L
- Establish functional sensitivity independent of the manufacturer by using Guideline 20
- There is no scientific justification to reflex from a less sensitive to a more sensitive test. (Insensitivity causes falsely high, not falsely low, values that are missed by reflex testing!)

Guideline 22. TSH Reference Intervals

TSH reference intervals should be established from the 95% confidence limits of the log-transformed values of at least 120 rigorously screened normal euthyroid volunteers who have:

- No detectable thyroid autoantibodies, thyroperoxidase antibodies (TPOAb) or thyroglobulin antibodies (TgAb) (measured by sensitive immunoassay)
- No personal or family history of thyroid dysfunction
- No visible or palpable goiter
- No medications (except estrogen).

Clinical Use of Serum TSH Measurements

Guideline 23. Levothyroxine (L-T4) Replacement Therapy for Primary Hypothyroidism

- L-T4, not desiccated thyroid, is the preferred medication for long-term replacement therapy for hypothyroidism.
- A euthyroid state is usually achieved with an average L-T4 dose of 1.6 micrograms/kg body weight/day. The initial dose and time to achieve full replacement should be individualized relative to age, weight and cardiac status. An initial L-T4 dose is normally 50-100 micrograms daily. Serum TSH measurement after six weeks will indicate the need for dose adjustment by 25-50 microgram increments.
- Children require higher doses of L-T4, up to 4.0 micrograms/kg bw/day, due to rapid metabolism. Serum TSH and FT4 values should be assessed using age-specific and method-specific reference ranges (see Table 3 in the original guideline document).
- A serum TSH level between 0.5 and 2.0 mIU/L is generally considered the optimal therapeutic target for the L-T4 replacement dose for primary hypothyroidism.
- TSH is slow to re-equilibrate to a new thyroxine status (see Guideline 2 above). Six to 8 weeks is needed before retesting TSH after changing the L-T4 dose or brand of thyroid medication.
- Intermittent or non-compliance with levothyroxine (L-T4) replacement therapy will result in discordant serum TSH and FT4 values (high TSH/high FT4) because of a persistently unstable thyroid state (see Guideline 2 above). Both TSH and FT4 should be used for monitoring such patients.
- Thyroxine requirements decline with age. Older individuals may require less than 1.0 microgram/kg bw/day and may need to be titrated slowly. Some physicians prefer to gradually titrate such patients. An initial dose of 25 micrograms is recommended for patients with evidence of ischemic heart disease followed by dose increments of 25 micrograms every 3-4 weeks until

the full replacement dose is achieved. Some believe that a higher target TSH (0.5-3.0 mIU/L) value may be appropriate for the elderly patient.
- In severe hypothyroidism an initial L-T4 loading dose is the most rapid means for restoring a therapeutic FT4 level because the excess of unoccupied binding sites may blunt the FT4 response to treatment.
- Thyroxine requirements increase during pregnancy. Thyroid status should be checked with TSH + FT4 during each trimester of pregnancy. The L-T4 dose should be increased (usually by 50 micrograms/day) to maintain a serum TSH between 0.5 and 2.0 mIU/L and a serum FT4 in the upper third of the normal reference interval.
- Post-menopausal women starting hormone replacement therapy may need an increase in their L-T4 dose to keep the serum TSH within the therapeutic target.
- TSH testing of patients receiving a stable L-T4 dose is recommended on an annual basis. The best time for TSH testing is not influenced by the time of day the L-T4 dose is ingested.
- Ideally L-T4 should be taken before eating, at the same time of day, and at least 4 hours apart from any other medications or vitamins. Bedtime dosing should be 2 hours after the last meal.
- Patients beginning chronic therapy with cholestyramine, ferrous sulfate, calcium carbonate, soy protein, sucralfate and antacids containing aluminum hydroxide that influence L-T4 absorption may require a larger L-T4 dose to maintain TSH within the therapeutic target range.
- Patients taking rifampin and anticonvulsants that influence the metabolism of L-T4 may also need an increased L-T4 dose to maintain the TSH within the therapeutic target range.

Guideline 24. Levothyroxine (L-T4) Suppression Therapy

- Serum TSH is considered a growth factor for differentiated thyroid cancer (DTC). The typical L-T4 dose used to suppress serum TSH in DTC patients is 2.1 micrograms/kg body weight/day.
- The target serum TSH level for L-T4 suppression therapy for DTC should be individualized relative to the patient's age and clinical status including cardiac factors and DTC recurrence risk.
- Many physicians use a serum TSH target value of 0.05-0.1 mIU/L for low-risk patients and a TSH of <0.01 mIU/L for high-risk patients.
- Some physicians use a low-normal range therapeutic target for TSH when patients have undetectable serum Tg levels and have had no recurrence 5-10 years after thyroidectomy.
- If iodide intake is sufficient, L-T4 suppression therapy is rarely an effective treatment strategy for reducing the size of goiters.
- Over time, multi-nodular goiters typically develop autonomy that is characterized by a subnormal serum TSH level. Serum TSH should be checked before initiating L-T4 suppression therapy in such patients.

Guideline 25. TSH Measurement in Hospitalized Patients

- TSH + T4 (FT4 or TT4) is the most useful test combination to detect thyroid dysfunction in a sick hospitalized patient.
- It is more appropriate to use a widened TSH reference interval (0.05 to 10.0 mIU/L) in the hospitalized setting. Serum TSH levels may become subnormal

transiently in the acute phase and become elevated in the recovery phase of an illness.

- A serum TSH value between 0.05 and 10.0 mIU/L is usually consistent with a euthyroid state, or only a minor thyroid abnormality that can be evaluated by retesting after the illness subsides. (This only applies to patients not receiving medications such as dopamine that directly inhibit pituitary TSH secretion.)
- A low-normal TSH level in the presence of a low TT4 and low TT3 may reflect central hypothyroidism as a result of a prolonged illness; whether or not this condition requires immediate treatment remains uncertain and is currently controversial.
- When thyroid dysfunction is suspected, a thyroid peroxidase antibody (TPOAb) test may be useful to differentiate autoimmune thyroid disease from nonthyroidal illness (NTI).

Guideline 26. Levothyroxine (L-T4) Replacement Therapy for Central Hypothyroidism

- A serum FT4 level in the upper third of the reference interval is the therapeutic target for the L-T4 replacement dose used to treat central hypothyroidism due to pituitary or hypothalamic dysfunction.
- When using FT4 as the therapeutic endpoint for central hypothyroidism, the daily dose of L-T4 should be withheld on the day of the FT4 measurement. (Serum FT4 is increased [~13 %] above baseline for 9 hours after ingesting L-T4).

Guideline 27. Clinical Utility of TSH Assays (Functional Sensitivity $\leq$0.02 mIU/L)

- Serum TSH measurement is the most diagnostically sensitive test for detecting mild (subclinical), as well as overt, primary hypo- or hyperthyroidism in ambulatory patients.
- The majority (>95%) of healthy euthyroid subjects have a serum TSH concentration below 2.5 mIU/L. Ambulatory patients with a serum TSH above 2.5 mIU/L when confirmed by a repeat TSH measurement made after 3-4 weeks, may be in the early stages of thyroid failure, especially if TPOAb is detected.
- A serum TSH measurement is the therapeutic endpoint for titrating the L-T4 replacement dose for primary hypothyroidism (see Guideline 23 above) and for monitoring L-T4 suppression therapy for differentiated thyroid carcinoma (see Guideline 24 above).
- Serum TSH measurements are more reliable than FT4 in hospitalized patients with non-thyroidal illness not receiving dopamine. Serum TSH should be used in conjunction with T4 (TT4 or FT4) testing for hospitalized patients (Guidelines 6 and 26 above).
- TSH cannot be used to diagnose central hypothyroidism because current TSH assays measure biologically inactive TSH isoforms.
- Central hypothyroidism is characterized by an inappropriate normal or slightly elevated serum TSH level and a blunted (<2-fold rise/ $\leq$4.0 mIU/L increment) TRH response.
- When the serum FT4 is low and yet the serum TSH is only minimally elevated (<10 mIU/L), a diagnosis of central hypothyroidism should be considered.

- Serum TSH measurements are an important pre-natal and first trimester screening test to detect mild (subclinical) hypothyroidism in the mother (see Guideline 4 above).
- A low TSH in the setting of a multinodular goiter suggests the presence of mild (subclinical) hyperthyroidism due to thyroid autonomy.
- A serum TSH measurement is required for confirming that an elevated thyroid hormone level is due to hyperthyroidism and not a thyroid hormone binding protein abnormality (such as FDH).
- A serum TSH measurement is the primary test for detecting amiodarone-induced thyroid dysfunction (see Guideline 5 above).

Guideline 28. For Manufacturers of TSH Tests

- Manufacturers that market TSH tests with differing sensitivities are urged to discontinue marketing the less sensitive product.
- There is no justification for the pricing of TSH assays to be based on sensitivity!
- There is no scientific justification for reflexing from a less sensitive to a more sensitive TSH test.
- Manufacturers should help laboratories independently establish functional sensitivity by providing appropriately low TSH human serum pools when requested.
- Manufacturers should indicate the use of calibration factors, especially if calibration factors are country-dependent.
- Manufacturers should quote the recovery of TSH Reference Preparation at the claimed functional sensitivity.
- Kit Package Inserts should:
    - Document the realistic functional sensitivity of the method using Guideline 20 protocol
    - Cite the functional sensitivity that can be achieved across a range of clinical laboratories
    - Display the typical between-run precision profile expected for a clinical laboratory
    - Recommend the use of functional sensitivity not analytical sensitivity to determine the lowest reporting limit. (Analytical sensitivity prompts laboratories to adopt an unrealistic detection limit.)

Thyroid Autoantibodies (TPOAb, TgAb, and TRAb)

Guideline 29. Thyroid Antibody Method Sensitivity and Specificity Differences

- Recognize and understand that the results of thyroid antibody tests are method-dependent.
- Thyroid antibody methods recognize different epitopes in the heterogeneous antibody populations present in serum.
- Thyroid antibody assay differences reflect different receptor preparations (receptor assays) or cells (bioassays) used in the assay.
- Assay differences can result from contamination of the antigen reagent with other autoantigens.
- Assay differences can result from the inherent assay design (i.e. competitive versus non-competitive immunoassay) as well as the signal used.

- Assay differences can result from the use of different secondary standards.

Guideline 30. Functional Sensitivity of Thyroid Antibody Tests

Functional sensitivity assessment of thyroid autoantibody tests should:

- Be determined with human serum pools containing a low autoantibody concentration
- Be determined using the same protocol as described for TSH (see Guideline 20 above) but with the between-run precision assessment made over a 6 to 12 month time-period to represent the appropriate clinical assessment interval.

Guideline 31. For Manufacturers Standardizing Thyroid Antibody Assays

- Assays should be standardized against MRC International Reference Preparations: MRC 65/93 for TgAb, MRC 66/387 for TPOAb and MRC 90/672 for TRAb
- New International Reference Preparations should be prepared for TgAb and TPOAb.
- Secondary standards should be fully characterized to avoid bias between different methods.
- Reference preparations or recombinant antigen preparations should be used when available.

Guideline 32. Preferred TPOAb Methodology

- Sensitive, specific TPOAb immunoassays, using suitable preparations of highly purified native or recombinant human TPO as the antigen, should replace the older insensitive, semi-quantitative anti-microsomal antibody (AMA) agglutination tests. (Consensus Level 90%*)
- The clinical significance of a low TPOAb concentration requires more study.

*The guideline recommendations are based upon expert consensus at a level of >95%, unless otherwise indicated.

Guideline 33. Reference Intervals for Thyroid Antibody Tests

Reference intervals for thyroid antibody tests should be established from 120 "Normal" subjects free from any history of thyroid disease: Subject selection should minimize the inclusion of persons with a predisposition for autoimmune thyroid disease. Normal subjects should be:

- Male
- Young (< 30 years of age)
- Have serum TSH levels between 0.5 and 2.0 mIU/L
- No goiter
- No personal or family history of thyroid disease
- No non-thyroid autoimmune diseases (e.g. lupus or diabetes)

Guideline 34. Recommended Uses for TPOAb Measurement

- Diagnosis of Autoimmune Thyroid Disease
- Risk factor for Autoimmune Thyroid Disease
- Risk factor for hypothyroidism during Interferon alpha, Interleukin-2 or Lithium therapy
- Risk factor for thyroid dysfunction during amiodarone therapy (see Guideline 5 above)
- Risk factor for hypothyroidism in Down's Syndrome patients
- Risk factor for thyroid dysfunction during pregnancy and for post-partum thyroiditis
- Risk factor for miscarriage and in-vitro fertilization failure

Guideline 35. For Manufacturers Developing TgAb Methods

The epitope specificity of thyroid autoantibody (TgAb) methods should be broad not restricted, since TgAb epitope specificity may be wider for TgAb-positive patients with DTC compared to patients with autoimmune thyroid disease.

Guideline 36. TgAb Measurement in Non-Neoplastic Conditions

- In iodide sufficient areas, it is not usually necessary or cost-effective to order both TPOAb and TgAb, because TPOAb-negative patients with detectable TgAb rarely display thyroid dysfunction.
- In iodide deficient areas, serum TgAb measurements may be useful for detecting autoimmune thyroid disease when patients have a nodular goiter.
- Monitoring iodide therapy for endemic goiter.

Guideline 37. TgAb Measurement in Differentiated Thyroid Carcinomas (DTCs)

The TgAb concentration should be measured in ALL patient sera prior to Tg analysis because low levels of TgAb can interfere with serum Tg measurements causing either falsely low or undetectable or high values depending on the Tg method used.

- TgAb should be measured in every serum specimen sent to the laboratory for Tg testing.
- Serial TgAb measurements should be made on all TgAb-positive DTC patients using the same manufacturer's method because serial TgAb values have prognostic significance for monitoring response to DTC treatment.
- TgAb methods should be immunoassay not agglutination, because low levels of TgAb can interfere with serum Tg measurements made by most methods, and serial measurements must be quantitative not qualitative.
- Serum Tg recovery tests do not reliably detect the presence of TgAb and should be discouraged as a method for detecting TgAb (see Guideline 46 below).
- Before changing the TgAb method, the laboratory should inform physician users and evaluate the relationship between the old and proposed new method values. Patients should be re-baselined if the difference between the methods is >10% coefficient of variation (CV).

Guideline 38. TSH Receptor Antibody (TRAb) Tests

Clinical laboratory TRAb assays are either:

- Receptor or TSH binding inhibition tests (TBII) that do not measure stimulatory activity directly but detect factors in the serum specimen that block the binding of a labeled TSH preparation to an in-vitro TSH receptor preparation. These tests are the more commonly used TRAb assays in clinical laboratories.
- TSH receptor bioassays (TSAb) use cells (FRTL-5 cells, or more recently CHO transfected with human TSH receptor) to detect thyroid stimulating immunoglobulins (TSAb) that either stimulate cAMP (cyclic adenosine monophosphate) or iodide uptake. These tests are not routinely available in all countries.
- In general, there is a poor correlation between TSAb and TBII results (60-75%). TSAb assays claim to be positive in 80-100% and TBII assays positive in 70 to 90% of untreated Graves' hyperthyroid patients. Neither test has high specificity or sensitivity for predicting remission from Graves' hyperthyroidism.
- Normal hCG as well as abnormal hCG production in choriocarcinoma are known to interact with the TSH receptor which could lead to false positive results. This might be observed in rare cases of choriocarcinoma but not in normal pregnancy or treated hydatiform mole in which the level of hCG is not high enough to cause a false positive result.

Guideline 39. Clinical Uses of TRAb Measurement

- To investigate the etiology of hyperthyroidism when the diagnosis is not clinically obvious.
- A declining TRAb concentration during long-term antithyroid drug therapy is suggestive of remission. However, TRAb measurements can be misleading in 25% of such patients.
- TRAb measurements are useful to diagnose Graves' disease patients and for relating TRAb values to a treatment algorithm.
- To evaluate patients suspected of "euthyroid Graves' ophthalmopathy". Undetectable TRAb, however, does not exclude the condition.
- Although TSAb assays have theoretical advantages, some believe that TBII tests which detect both stimulating (TSAb) and the rare cases of blocking (TBAb/TSBAb) antibodies are equally useful.
- For pregnant women with a past or present history of Graves' disease. Note: Pregnant women who are euthyroid after receiving prior antithyroid drug treatment for Graves' disease have a negligible risk for fetal or neonatal hyperthyroidism.
- Euthyroid pregnant women (± L-T4 treatment) who have had prior radioiodide treatment for Graves' disease should have TRAb measured both early in pregnancy when a high value is a risk factor for fetal hyperthyroidism (2-10%), and during the third trimester to evaluate the risk of neonatal hyperthyroidism.
- Pregnant women who take antithyroid drugs (ATDs) for Graves' disease to maintain a euthyroid state during pregnancy should have TRAb measured in the third trimester. A high TBII value should prompt a clinical and biochemical evaluation of the neonate for hyperthyroidism, both at birth (cord blood) and at 4-7 days after the effects of transplacental passage of ATD have been lost.

- The assessment of the risk of fetal and neonatal thyroid dysfunction necessitates the detection of either blocking or stimulating TRAb when mothers have no intact thyroid following past therapy for Graves' hyperthyroidism.
- To identify neonates with transient hypothyroidism due to the presence of TSH receptor blocking antibodies.

Guideline 40. Improvements Needed in Thyroid Antibody Tests

- Current thyroid autoantibody assays should be submitted to a comparative study of their analytical and clinical performances.
- A comparison study of the antigen preparations currently in use would facilitate the identification of the method(s) best suited for clinical thyroid autoantibody testing.
- The characteristics of the antigen preparations used in the test should be stated for all thyroid autoantibody assays.
- Reference preparations of antigens should be made available.

Guideline 41. For Manufacturers Developing Thyroid Antibody Tests

- Absolute or "gold standard" methods remain a target for the future.
- The kit package insert should document the methods used to produce the antigen reagents, the assay design and all experimental conditions affecting the antigen-antibody interactions.
- The specificity of the secondary standards should be selected relative to the interactions between the autoantibodies in patient sera and their specific antigen.
- TPOAb and TgAb immunometric assays (IMAs) should be checked for hook effects using ~20 specimens with antibody concentrations >1,000 kIU/L and ~20 specimens with values above 10,000 kIU/L.
- TgAb methods should be checked for high antigen (Tg) effects by spiking a range of sera containing low TgAb concentration to Tg levels >10,000 micrograms/L (ng/ml) and >100,000 micrograms/L (ng/ml).

Thyroglobulin (Tg)

Guideline 42. For Manufacturers Developing Tg Methods

The diluent used for standards should ideally be Tg-free/TgAb-free human serum. Non-serum matrices should be selected to produce a signal (radioactive counts, relative light units etc) that is identical to Tg-free/TgAb-free human serum to avoid matrix-related biases.

Guideline 43. For Laboratories Considering Changing their Tg Method

Select a Tg method on the basis of its performance characteristics not cost or expediency. Before changing the Tg method the laboratory should consult with physician users and compare results between the old and proposed new method using specimens from both TgAb-negative and TgAb-positive patients.

- TgAb-negative patients: If the bias between the old and new method results is > 10%, physicians should be informed and given sufficient time to re-baseline critical patients.
- TgAb-positive patients: The laboratory should warn physicians about the likely direction of interference in the presence of TgAb.
- If serum Tg values are to be reported for TgAb-positive specimens, an appropriate cautionary comment should be displayed on each laboratory report.
- FOR IMMUNOMETRIC (IMA) METHODS:

  IMA methods may give inappropriately low or underestimate serum Tg levels when TgAb is present. Undetectable serum Tg results cannot be used to indicate the absence of tumor in a TgAb-positive patient. A detectable Tg level indicates that Tg is present, but concentrations may be underestimated.

- FOR RADIOIMMUNOASSAY (RIA) METHODS:

  RIA methods may give inappropriately higher- or underestimated serum Tg values when TgAb is present (depending on the method). Detectable serum Tg results should not be used as the sole factor for determining the presence of residual thyroid tissue or tumor.

Guideline 44. Tg Assay Functional Sensitivity & Between-Run Precision

Functional sensitivity and between-run precision should be established using the same protocol as for TSH (Guideline 20) with three important stipulations:

- Use human serum pools that contain no TgAb, determined by a sensitive TgAb immunoassay.
- Target values are recommended for low, medium and high pools:

  Low Pool (used to determine functional sensitivity) should have a serum Tg value that is 30 to 50 % higher than the expected functional sensitivity (FS) limit.

  (If FS = 1.0 microgram/L [ng/ml] the low pool target should be 1.3 to 1.5 micrograms/L [ng/ml])

  Medium Pool target = ~10 micrograms/L (ng/ml), i.e. close to the mid-normal range.

  High Pool target = ~90% of the upper reportable limit suggested by manufacturer.

- The test period used for assessing between-run precision should be at least 6 months. This is more representative of the clinical interval used for monitoring DTC patients than the 6-8 week interval recommended for TSH in Guideline 20.

Guideline 45. Testing for "Hook" Effects

- A two-step design is recommended to minimize hook problems. "One-step" assays that are more prone to hook effects should measure every specimen at two dilutions (undiluted and 1:10) to check a discrepancy in the two results.
- All assays (two-step or one-step) should be validated for a hook effect before manufacturer release.
- To check for a hook effect, measure serial 10-fold dilutions of ~ 20 different TgAb-negative specimens with serum Tg concentrations above 10,000 micrograms/L (ng/ml) and ~ 20 different TgAb-negative specimens with serum Tg values above 100,000 micrograms/L until parallelism is demonstrated.

Guideline 46. TgAb Interference and Recovery Tests

- Recovery tests do not reliably detect TgAb and should be discouraged and eliminated. Previous studies have shown that low recoveries sometimes seen in the absence of TgAb were flawed by the insensitivity of early TgAb methods. When sensitive immunoassays are used, TgAb can always be detected when recovery is low.
- Discordance between IMA and RIA Tg measurements for TgAb-positive specimens suggests TgAb interference (if values are typically concordant for TgAb-negative specimens).
- Laboratories should not report undetectable serum Tg values for TgAb-positive patients if the method produces inappropriately low or undetectable serum Tg values for TgAb-positive DTC patients with documented disease.

Guideline 47. For Manufacturers and Laboratories

Tg method package inserts should cite realistic performance characteristics for the method (i.e. performance that can be reproduced across a range of clinical laboratories).

- Assays should be standardized against the CRM-457 reference preparation. Assays <u>not</u> standardized against CRM-457 should provide a correction factor.
- The mean Tg level and the 2sd limits of the reference range for TgAb-negative normal euthyroid subjects (established using Guideline 48 below) should be cited in all publications to allow comparison of absolute values.
- Assays that cannot detect Tg in all normal sera have suboptimal sensitivity for monitoring DTC patients.
- The matrix used to dilute the standards should be checked for bias (see Guideline 42 above).
- Functional sensitivity and within and between-run precision should be established using the protocols described in Guideline 44, above.
- TgAb interference should be assessed by checking for RIA:IMA discordances in TgAb-positive sera (TgAb levels 100 to >1000 kIU/L [IU/ml]).
- TgAb immunoassay measurements and <u>not</u> exogenous Tg recovery studies should be used to detect TgAb interference (see Guideline 46 above).
- Serum Tg values for TgAb-positive specimens should not be reported if the method gives inappropriately undetectable values in TgAb-positive DTC patients with documented disease.

Guideline 48. Serum Tg Normal Reference Intervals

- Tg reference ranges should be determined locally because serum Tg concentrations are influenced by iodide intake:

  Countries with adequate iodide intake: The serum Tg reference interval for a TgAb-negative euthyroid population using CRM-457-standards approximates 3 to 40 micrograms/L (ng/ml).

  Countries manifesting iodide deficiency: The population mean Tg value and the upper Tg reference limit may be elevated relative to the degree of iodide deficiency.

- Laboratories should validate their Tg normal reference interval independent of the manufacturer.
- Tg reference ranges should be established from the log transformed values of 120 normal, non-smoking, euthyroid (TSH 0.5 to 2.0 mIU/L) subjects less than 40 years of age with no personal or family history of thyroid disease and with no evidence of TgAb or TPOAb.
- It is misleading to cite the normal euthyroid reference range when reporting serum Tg values for thyroidectomized DTC patients. Reference values should be related to the euthyroid reference limits for the method, the thyroid mass and TSH status.

  For example, the reference ranges below would be appropriate for a Tg method with a euthyroid reference range of 3-40 micrograms/L (ng/ml):

  Tg microgram/L (ng/ml): 3-40
  Condition: Normal thyroid gland reference (TSH 0.4-4.0 mU/L)

  Tg microgram/L (ng/ml): 1.5-20
  Condition: Normal thyroid gland reference (TSH <0.1 mU/L)

  Tg microgram/L (ng/ml): <10
  Condition: Thyroid lobectomy (TSH <0.1 mU/L)

  Tg microgram/L (ng/ml): <2
  Condition: Near-total thyroidectomy (TSH <0.1 mU/L)

Guideline 49. Serum Tg Measurement for Non-Neoplastic Conditions

Abnormally high serum Tg concentrations result from abnormalities in thyroid mass, excessive thyroidal stimulation, or physical damage to the thyroid secondary to surgery, fine needle aspiration (FNA) or thyroiditis. Serum Tg measurements are useful:

- For diagnosing thyrotoxicosis factitia which is characterized by a non-elevated serum Tg.
- To investigate the etiology of congenital hypothyroidism in infants detected by neonatal screening.
- To assess the activity of inflammatory thyroiditis, e.g., subacute thyroiditis, or amiodarone-induced thyroiditis.

Guideline 50. Serum Tg Measurements for Differentiated Thyroid Carcinoma (DTC)

TgAb-Negative Patients:

- Pre-operative serum values (drawn before or >2 weeks after FNA) are useful for determining the Tg-secretion capacity of the tumor.
- The acute post-operative decline in serum Tg reflects the completeness of surgery with the serum Tg half-life of 3-4 days. (If thyroid hormone is given to prevent a rise in TSH).
- There is no "normal range" for a thyroidectomized patient! Completely athyreotic patients should have no Tg detectable in their serum, even if the TSH is elevated.
- Useful reference point: one gram of normal thyroid tissue releases ~1 microgram/L (ng/ml) Tg into the serum when TSH is normal, and ~0.5 microgram/L (ng/ml) when TSH is suppressed <0.1 mU/L.
- When serum Tg is <u>detectable</u> during L-T4 treatment (stable TSH), changes in tumor burden can be monitored by serial serum Tg measurements without thyroid hormone withdrawal or rhTSH.
- When serum Tg is <u>undetectable</u> during L-T4 treatment (and TgAb is absent) a TSH-stimulated serum Tg is more sensitive for detecting disease localized to the neck than serum Tg measured during TSH suppression.
- There is typically a >5-fold increase in serum Tg above basal L-T4 Rx. values following TSH stimulation (endogenous or rhTSH). Paired studies show that rhTSH-stimulated Tg responses are approximately half those seen with endogenous TSH following thyroid hormone withdrawal.

TgAb-Positive Patients:

- Typically display blunted or absent TSH-stimulated serum Tg responses.
- Serial TgAb measurements (by immunoassay) are valuable as a surrogate tumor marker test

Calcitonin (CT) and RET Proto-oncogene

Guideline 51. Calcitonin (CT) Assays

- Mature (32 amino acid) CT is the principal tumor marker for medullary thyroid carcinoma (MTC).
- CT measurements used for the diagnosis of MTC and for monitoring purposes should be performed using two-site immunometric assays that are specific for the mature 32 amino acid monomer of CT.
- Currently, the lower normal threshold for CT is generally accepted as being under 10 pg/ml (ng/L).
- As new, more sensitive CT kits become available, the lower CT threshold should be redefined.

Guideline 52. Clinical Utility of Serum Calcitonin Measurements for Diagnosing Medullary Thyroid Carcinoma

- Calcitonin (CT) measurements are method-dependent. This can impact the interpretation of CT results.
- Increased levels of calcitonin in the serum can be seen for patients with autoimmune thyroid diseases (Hashimoto's thyroiditis or Graves' disease).
- Hyperplasia of the C cells (HCC) is the earliest histological finding prior to the development of a microcarcinoma. A non-elevated CT may be seen with HCC in the earliest stages of developing medullary thyroid carcinoma (MTC).
- A rise in serum CT levels above 10 pg/ml (ng/L) suggests early MTC at the microcarcinoma stage
- There is a positive correlation between CT levels and tumor mass.

Guideline 53. Postoperative Follow-up of MTC

- Serum CT and carcino-embryonic antigen (CEA) should be measured just prior to, and 6 months after, surgery for MTC. Serum CT levels fall slowly in some patients. The first post-operative CT measurement should not be made until 2 weeks after surgery.
- The presence of residual tissue or a recurrence of MTC can only be ruled out if both basal and post pentagastrin or calcium-stimulated CT levels are undetectable.

Guideline 54. Genetic Risk of MTC

- In multiple endocrine neoplasia (MEN) 2 kindred 50% of the family members are potentially affected by the disease.
- Almost all patients bearing RET mutations will develop MTC. (Note: inactivating mutations of the ret gene also cause Hirschsprung's disease).
- 5-10% of sporadic MTC have been found to carry germline RET mutations. Therefore RET analysis is justified in all patients with apparently sporadic MTC.

Urinary Iodine Measurement

Guideline 55. Urinary Iodine Measurement

- The Technicon Autoanalyser is generally no longer commercially available, with the result that laboratories seeking to commence iodide measurement will need to develop manual in-house methods.
- Mass spectrometry is a simple and reproducible method which can be recommended if such equipment is already on site.
- Many simplified digestion methods incorporating Sandell-Kolthoff (SK) colorimetry have been described.
- Wet-ashing reagents perchloric acid and potassium chlorate are potentially explosive and their use requires availability of an expensive fume hood. A less hazardous system using ammonium persulfate may be preferable.
- Measurement of iodide in samples other than urine (e.g., tissues, foodstuffs) may still require the more conventional dry or wet-ashing techniques.
- Inter and intra assay coefficient of variation (CV) should be < 10% and recovery of added iodide should be between 90 and 100%.
- In industrialized countries, clinical laboratories are most frequently requested to perform urinary iodide measurements to investigate iodide overload. One

of the simplified methods outlined above, or a semi-quantitative kit is the method of choice.
- To facilitate uniformity in concentration units used to report urinary iodide excretion, UI should be expressed as microgram Iodide/L of urine (microgram/L).

Thyroid Fine Needle Aspiration (FNA) and Cytology

Guideline 56. Use of Fine Needle Aspiration (FNA) of the Thyroid

- FNA is recommended for all palpable solitary or dominant nodules, independent of size.
- FNA is preferred over thyroid scan or ultrasonography as the initial diagnostic test for thyroid nodules. However, a previous ultrasound may aid the physician performing the aspiration.
- When TSH is suppressed or the patient is thyrotoxic, a nuclear scan may be indicated before FNA. However, the result of the scan should not exclude the necessity for FNA.
- "Hot" nodules detected by nuclear scan are less likely to be malignant than "cold" nodules.

Guideline 57. For Physicians

- It is important that the endocrinologist, surgeon, nuclear medicine physician and cytopathologist act in concert to integrate the staging information into a long-term treatment plan and thereby ensure continuity of care.
- Preferably, the physicians responsible for the long-term management of the patient should review the slides with the cytopathologist and understand the cytopathologic interpretation to establish meaningful treatment strategies for the patient.

Guideline 58. Selection of Physicians to Perform FNA

Thyroid gland aspirations should be performed by physicians who:

- Are skilled in the technique and perform thyroid aspirations frequently.
- Can understand the interpretation of the cytology results.
- Are able to recommend appropriate therapy depending on the results of the aspiration.

Guideline 59. Selection of the Cytopathologist

- The cytopathologist should have an interest and experience in reading thyroid cytology. If an experienced cytopathologist is not available locally, the slides should be sent for review by a cytopathologist with thyroid expertise outside the institution.
- Cytopathologists should be willing to review the slides with the patient's physician on request.

Guideline 60. Cytopathologic Characteristics

Thyroid cytology interpretation can be difficult and challenging. The amount of tissue contained on the slides may depend on the method of aspiration (ultrasound versus manual). The evaluation should assess:

- The presence or absence of follicles (microfollicles versus variable-sized follicles)
- Cell size (uniform versus variable)
- Staining characteristics of the cells
- Tissue polarity (cell block only)
- Presence of nuclear grooves and/or nuclear clearing
- Presence of nucleoli
- Presence and type of colloid (watery and free versus thick and viscous)
- Monotonous population of either Follicular or Hurthle cells
- Presence of lymphocytes

Guideline 61. For Laboratories and Physicians

- In addition to routine cytology, the laboratory should provide access to special immunoperoxidase staining for calcitonin (CT), thyroglobulin (Tg), thyroid peroxidase (TPO) or Galectin-3 for special cases. (Send out to a different laboratory if necessary).
- Laboratories should archive all slides and tissue blocks "in trust" for the patient and make materials available for a second opinion when requested.
- Cytopathology laboratories should use standardized reporting of FNAs. The simplest approach uses four diagnostic categories: (1) Benign, (2) Malignant, (3) Indeterminate/Suspicious, and (4) Unsatisfactory/Inadequate. This should help achieve meaningful comparisons among different laboratories regarding outcomes.
- Cytopathology laboratories should share their analysis of FNA results with clinicians by citing their rates for true and false positives and negatives.

Guideline 62. Follow-up of Patients with Benign Disease

- Some advocate performing a second FNA several months later to confirm the test.
- Others do not recommend a repeat FNA if the first yielded adequate tissue, provided that the nodule was less than 2 cm and has been stable in size during a year of follow up. In this case, follow-up with an annual physical examination and measurement of the nodule size, preferably with ultrasound, is recommended. If ultrasound is not available, changes in nodule size may be detected by measurements made by a tape and/or ruler.
- It is recommended that enlarging lesions or any clinically suspicious nodules should be re-aspirated.

Guideline 63. Patients with Inadequate or Non-diagnostic FNA

- Repeat FNA for small nodules often yields adequate cellular material for a diagnosis. Preferably, the repeat FNA should be done with ultrasound guidance. FNA using ultrasound guidance reduces the incidence of inadequate specimens from 15-20% down to 3-4%.
- Ultrasound guided FNA is also indicated for nodules <1.5 cm, cystic (complex) nodules to assure sampling of the solid component, posterior or high

substernal nodules or any nodule greater than 1.0 cm that is difficult to palpate, especially in the obese, muscular or large frame patient. The principal (i.e dominant) nodule(s) in a multinodular goiter should be biopsied using ultrasound guidance.

Screening for Congenital Hypothyroidism

Guideline 64. Laboratories Performing Neonatal Screening for Congenital Hypothyroidism

Only laboratories with experience in automated immunoassay procedures, information technology and with computer back-up, and appropriately trained staff, should undertake high volume screening for Congenital Hypothyroidism.

Guideline 65. For Laboratories Performing Thyroid Testing of Neonates and Infants

- Thyroid test results in neonates must be reported with gestation and age-specific reference intervals, respectively.
- Each Laboratory should establish its own cut off levels according to the method used.

Guideline 66. Pre-term and Early Discharge of Neonates

The TSH surge that follows the cutting of the umbilical cord and lasts for the first 24 hours may be delayed in pre-term infants and may lead to more false-positive TSH results when infants are tested within 24 hours of birth.

- When using TSH to screen pre-term infants, a second sample collected 2 to 4 weeks after birth is recommended, since in some cases there is a delayed rise in TSH, perhaps due to immaturity of the pituitary-thyroid feedback mechanism.
- The TT4--first approach may offer advantages for very low birth weight infants or when screening can only be performed within 24 hours of birth.

Guideline 67. Countries with Iodine Deficiency

Primary TSH testing is recommended in preference to primary TT4 with reflex TSH in countries that have mild or moderate iodine deficiency.

Guideline 68. Performance Criteria for Blood Spot TSH Screening of Newborns

- Functional sensitivity of the TSH assay should be at least 1.0 mIU/L.
- Between run coefficient of variation should ideally be <10% and not more than 20%.
- Internal quality control samples should cover the reportable range and must be included in every run.
- At least one of the quality controls materials should be supplied by a different manufacturer from the TSH reagent manufacturer.
- Standards should be made in blood, i.e. be identical to specimens tested.

- Use the same filter paper for the samples, standards and controls.
- Participation in National and/or International external quality control programs is essential (see Appendix B in the original guideline document).

Guideline 69. TSH Cut-off values for the Screening of Neonates > 48 hours of age

Reported values should be identified in whole blood or serum units. It is necessary to increase the whole blood units by 30-50% to approximate serum units.

- Initial blood spot TSH <10 mIU/L whole blood units--no further action
- Initial blood spot TSH 10-20 mIU/L whole blood units--repeat the test on a second blood spot
- Initial blood spot TSH >20 mIU/L whole blood units--recall infant for evaluation by pediatric endocrinologist

Guideline 70. Filter Paper Eluate Measurements

Measurements made on filter paper eluates are not diagnostic. Values are at best only semi-quantitative and help identify individuals likely affected by congenital hypothyroidism. Any abnormal newborn screening result must be confirmed with quantitative serum thyroid tests.

Guideline 71. Confirmation Testing for Abnormal Screening Tests (TT4 or TSH)

- Confirmatory blood samples from the neonate should be drawn by venipuncture.
- Some programs in Europe advocate follow-up testing of only the infant and in some cases the thyroid status of the mother is also investigated using serum FT4, TSH and TPOAb testing.
- Check the mother for TSH receptor blocking antibodies.
- Use method and age-specific reference intervals for TT4 and TSH testing of neonates.

Guideline 72. Detection of Transient Congenital Hypothyroidism (CH)

Since CH may be transient as a result of transplacental passage of TSH receptor blocking antibodies, it is recommended that the diagnosis be re-evaluated in all cases at 2 years of age.

At 2 years of age a blood specimen should be obtained for basal serum FT4/TSH measurements. Discontinue L-T4 treatment and retest serum FT4/TSH after 2 weeks and again after 3 weeks. Almost 100% of children with true CH have elevated TSH levels after 2 weeks off of treatment.

Guideline 73. Treatment and Follow-up of Infants with Congenital Hypothyroidism

- In Europe, a flat L-T4 dose of 50 micrograms/day is used to minimize the risk of overtreatment as compared with more frequent dose changes.

- In the USA, treatment is typically initiated with L-T4 at a dose of 10-15 micrograms/kg/day. The goal is to raise the circulating T4 above 10 micrograms/dl by the end of the first week.
- During the first year of life, TT4 is usually maintained in the upper half of the normal reference range (therapeutic target 10-16 micrograms/dl/127-203 nmol/L) or if FT4 is used, the therapeutic target is between 1.4 and 2.3 ng/dl (18 and 30 pmol/L) depending on the reference range (see Table 3 in the original guideline document).
- Infants and children diagnosed with congenital hypothyroidism should be monitored frequently in the first two years of life using serum TSH as the primary monitoring test with FT4 as the secondary parameter, employing age-appropriate reference intervals.
- Monitoring should be every 1-2 months during the first year or life, every 1-3 months during the second and third years and every 3-6 months until growth is complete.
- If circulating T4 levels remain persistently low and the TSH remains high despite progressively larger replacement doses of L-T4, it is important to first eliminate the possibility of poor compliance.
- The most frequent reason for failure to respond to replacement therapy has been interference with adsorption by soy-based formulas. L-T4 should not be administered in combination with any soy-based substances or with medications that contain iron.

Guideline 74. For Physicians

- Repeat tests when the clinical picture conflicts with the laboratory test results!
- Potential pitfalls in screening are ubiquitous and no laboratory is immune!
- Maintain a high degree of vigilance. Despite all safeguards and automated systems, screening programs will occasionally miss infants with congenital hypothyroidism. Do not be lulled into a false sense of security by a laboratory report bearing normal thyroid function values.

The Importance of the Laboratory-Physician Interface

Guideline 75. For Laboratories and Physicians

- It is essential that clinical laboratory scientists develop an active collaboration with the physicians using their laboratory services in order to select thyroid tests with the most appropriate characteristics to serve the patient population in question.
- An active laboratory-physician interface ensures that high quality, cost-effective assays are used in a logical sequence, to assess abnormal thyroid disease presentations and to investigate discordant thyroid test results.

Guideline 76. Patients' "Bill of Rights"

- Physicians should have the right to send specimens for testing to non-contracted laboratories when they can show that the contracted laboratory thyroid test results are not diagnostically valid or relevant.
- Physicians should have the right to request their laboratory to send a specimen to another laboratory for testing by a different manufacturer's method if the test results are in disagreement with the clinical presentation.

Guideline 77. For Manufacturers

Manufacturers should cooperate closely with laboratories using their products. Manufacturers should:

- Rapidly inform all users of reagent problems and method interferences and recommend how to minimize their clinical impact.
- The composition of assay kits should not be changed, even if the goal is to reduce interference, without first informing customers. If the procedure has to be changed, the change should be indicated on the label of the kit (i.e. by a version number).

Guideline 78. For Laboratories

- Every clinical laboratory should develop a relationship with another laboratory that uses a different manufacturer's method. Re-measurement of specimens with discordant results by an alternative method is the cornerstone of investigating whether a discordant result is caused by an interfering substance present in the specimen or as a result of "true" disease (see Table 1 in the original guideline document).
- Laboratories should be able to provide physicians with the details of the thyroid method principles underpinning the test being used together with functional sensitivity, between-run precision, interferences and any bias relative to the method or other methods, and whether the tests are performed locally or sent to a reference laboratory.

Guideline 79. Misinterpretations that Can Lead to Serious Errors

When physicians or laboratorians are not aware of the limitations of test methods, serious medical errors can result:

- Inappropriate thyroid ablation because high thyroid hormone levels were reported as a result of familial dysalbuminemic hyperthyroxinemia (FDH), the presence of thyroid hormone autoantibodies or thyroid hormone resistance.
- A missed diagnosis of T3-toxicosis in a frail elderly patient with nonthyroidal illness (NTI).
- Inappropriate treatment of a hospitalized patient for hypo- or hyperthyroidism on the basis of abnormal thyroid tests caused by NTI or a drug-related interference.
- A missed diagnosis of central hypothyroidism because the immunoreactive TSH level was reported as normal due to the measurement of biologically inactive TSH isoforms.
- Failure to recognize recurrent or metastatic disease in a thyroid cancer patient because serum Tg was inappropriately low or undetectable due to TgAb interference or a "hook" effect with an IMA measurement.
- Inappropriate treatment for differentiated thyroid carcinoma (DTC) on the basis of an abnormally elevated serum Tg caused by TgAb interference with a Tg RIA method.
- Failure to recognize that neonatal thyrotoxicosis can be masked by transplacental passage of antithyroid drugs given to the mother for Graves' disease.

Glossary of Abbreviations

AIH = Amiodarone-Induced Hyperthyroidism
AITD = Autoimmune Thyroid Disease
ANS = 8-Anilino-1-Naphthalene-Sulphonic Acid
ATD = Anti-Thyroid Drug Treatment
CT = Calcitonin
CV = % Coefficient of Variation
DTC = Differentiated Thyroid Carcinoma
FDH = Familial Dysalbuminemic Hyperthyroxinemia
FFA = Free Fatty Acid
FMTC = Familial Medullary Thyroid Carcinomas
FNA = Fine Needle Aspiration
FT3 = Free T3
FT4 = Free T4
HCC = C-cell Hyperplasia
HCG = Human chorionic gonadotropin
IMA = Immunometric Assay
L-T4 = Levothyroxine
MEN = Multiple Endocrine Neoplasia
MTC = Medullary Thyroid Carcinoma
NTI = Nonthyroidal Illness
PBI = Protein-Bound Iodine
Pg = Pentagastrin
RT3 = Reverse T3
RET = RET Proto-oncogene
RIA = Radioimmunoassay
T4 = Thyroxine
T3 = Triiodothyronine
TBG = Thyroxine Binding Globulin
TBPA = Thyroxine Binding Prealbumin
TT4 = Total Thyroxine
TT3 = Total Triiodothyronine
TTR = Transthyretin
Tg = Thyroglobulin
TgAb = Thyroglobulin Autoantibody
TPO = Thyroid Peroxidase
TPOAb = Thyroid Peroxidase Autoantibody
TBAb/TSBAb = TSH Receptor Blocking Antibody
TBII = TSH Binding Inhibitory Immunoglobulins
TRAb = TSH Receptor Antibody
TSAb = Thyroid Stimulating Antibody
TSH = Thyroid Stimulating Hormone (Thyrotropin)
WHO = World Health Organization (WHO)

CLINICAL ALGORITHM(S)

The original guideline contains a clinical algorithm for the diagnosis/treatment for medullary thyroid carcinoma (MTC).

EVIDENCE SUPPORTING THE RECOMMENDATIONS

TYPE OF EVIDENCE SUPPORTING THE RECOMMENDATIONS

The type of supporting evidence is not specifically stated for each recommendation. The guideline recommendations are based upon expert consensus at a level of >95%, unless otherwise indicated.

BENEFITS/HARMS OF IMPLEMENTING THE GUIDELINE RECOMMENDATIONS

POTENTIAL BENEFITS

Appropriate laboratory performance and clinical use of thyroid tests in the diagnosis and monitoring of thyroid disease

POTENTIAL HARMS

Not stated

QUALIFYING STATEMENTS

QUALIFYING STATEMENTS

The material in this monograph represents the opinions of the editors and does not represent the official position of the National Academy of Clinical Biochemistry or any of the co-sponsoring organizations. The National Academy of Clinical Biochemistry is the official academy of the American Association for Clinical Chemistry.

IMPLEMENTATION OF THE GUIDELINE

DESCRIPTION OF IMPLEMENTATION STRATEGY

An implementation strategy was not provided.

INSTITUTE OF MEDICINE (IOM) NATIONAL HEALTHCARE QUALITY REPORT CATEGORIES

IOM CARE NEED

Living with Illness

IOM DOMAIN

Effectiveness

IDENTIFYING INFORMATION AND AVAILABILITY

BIBLIOGRAPHIC SOURCE(S)

National Academy of Clinical Biochemistry (NACB). NACB: laboratory support for the diagnosis and monitoring of thyroid disease. Washington (DC): National Academy of Clinical Biochemistry (NACB); 2002. 125 p. [495 references]

ADAPTATION

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GUIDELINE DEVELOPER(S)

National Academy of Clinical Biochemistry - Professional Association

GUIDELINE DEVELOPER COMMENT

The creation of this guideline was a collaborative effort involving many thyroid experts from a number of professional organizations concerned with thyroid disease: American Association of Clinical Endocrinologists (AACE), Asia & Oceania Thyroid Association (AOTA), American Thyroid Association (ATA), British Thyroid Association (BTA), European Thyroid Association (ETA) and the Latin American Thyroid Society (LATS).

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National Academy of Clinical Biochemistry Committee

COMPOSITION OF GROUP THAT AUTHORED THE GUIDELINE

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FINANCIAL DISCLOSURES/CONFLICTS OF INTEREST

Not stated

GUIDELINE STATUS

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GUIDELINE AVAILABILITY

Electronic copies: Available from the National Academy of Clinical Biochemistry (NACB) Web site:

- Word Format
- Portable Document Format (PDF)

Print copies: NACB publications are available through American Association for Clinical Chemistry (AACC) Press. To make a purchase or request a catalog, contact AACC Customer Service at 202-857-0717 or custserv@aacc.org.

AVAILABILITY OF COMPANION DOCUMENTS

None available

PATIENT RESOURCES

None available

NGC STATUS

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